

# (2E,4Z)-Decadienoic acid and (2E,4Z,7Z)-decatrienoic acid, two herbicidal metabolites from *Streptomyces viridochromogenes* Tü 6105<sup>†</sup>

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**Abstract:** Two fatty acids, (2E,4Z)-decadienoic acid and (2E,4Z,7Z)-decatrienoic acid, the latter being described for the first time as a natural product, were detected in the culture filtrate of *Streptomyces viridochromogenes* Tü 6105 by HPLC-diode array screening, purified by chromatographic methods and structurally elucidated by NMR techniques. Both metabolites show strong herbicidal activity against *Lemna minor* and *Lepidium sativum*. The herbicidal activities of the isolated compounds were compared with those of similar fatty acids and derivatives.

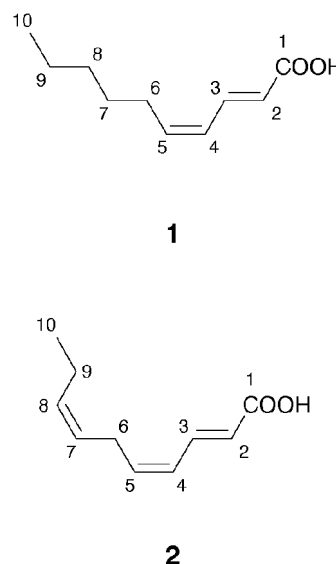
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**Keywords:** HPLC-diode array screening; *Streptomyces*; fermentation; structure elucidation; unsaturated fatty acids; herbicidal activity

## 1 INTRODUCTION

In a screening program for detection of novel secondary metabolites of freshly isolated actinomycete strains, extracts were analysed by a standardized reversed-phase HPLC procedure, diode array multi-wavelength monitoring (HPLC-DAD), and an HPLC-UV-Vis database which contains UV-visible spectra of about 540 reference compounds, mostly antibiotics.<sup>2</sup> An identification of a metabolite is given when retention time and UV-visible spectrum are identical. In parallel, extracts are tested for their biological activity in antibacterial, antifungal and herbicidal assays.

The analysis of the culture filtrate extract of strain Tü 6105 resulted in the identification of the well-known isoflavones genistein and daidzein. In addition, two further metabolites with congruent UV spectra were detected, which could not be identified by the HPLC-UV-Vis database, but were determined by NMR spectroscopic methods as (2E,4Z)-decadienoic acid (1) and (2E,4Z,7Z)-decatrienoic acid (2) (Fig 1).



**Figure 1.** (2E,4Z)-Decadienoic acid (1) and (2E,4Z,7Z)-decatrienoic acid (2), two unsaturated fatty acids with herbicidal activity.

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In this paper, the taxonomy of the producing strain, the fermentation and isolation of the fatty acids, as well as structure elucidation and their biological activities are described.

## 2 MATERIALS AND METHODS

### 2.1 Micro-organisms

Strain Tü 6105 was isolated from a soil sample collected near Cape Coast, Ghana, using lignin-vitamin-agar<sup>3</sup> with addition of cycloheximide (50 mg litre<sup>-1</sup>) and nalidixic acid (20 mg litre<sup>-1</sup>), and was identified according to Shirling and Gottlieb<sup>4,5</sup> as a strain of *Streptomyces viridochromogenes* (Krainsky) Waksman and Henrici. It is deposited in the culture collection of our institute in Tübingen.

Standard strains for testing the biological activity spectrum were obtained from ATCC, CBS, and the stock collection of the laboratory in Tübingen.

### 2.2 Fermentation and isolation

*S. viridochromogenes* Tü 6105 was cultivated in a 20-litre stirred tank fermentor (b20; Giovanola) using a production medium consisting of mannitol (20 g litre<sup>-1</sup>) and soybean meal (20 g litre<sup>-1</sup>) in tap water (pH 7.5). The fermentor was inoculated with 5% (v/v) of shaking cultures grown for 48 h in 500-ml Erlenmeyer flasks with one baffle on a rotary shaker at 120 rev min<sup>-1</sup> at 27°C in the same medium. The fermentation was carried out at 27°C for 26 h with an aeration rate of 0.5 v/v min<sup>-1</sup> and an agitation rate of 300 rev min<sup>-1</sup>.

Hyflo Super-cel (20 g litre<sup>-1</sup>) was added to the fermentation broth prior to separation of mycelium from culture filtrate by multiple sheet filtration. The culture filtrate was passed through an Amberlite XAD-16 column (10% resin volume relating to culture filtrate volume). Impurities were washed out with water and water+methanol (80+20 by volume); 1 and 2 were desorbed with water+methanol (20+80 by volume). After removing the organic solvent by evaporation under vacuum, the aqueous residue was adjusted to pH 7 and extracted three times with ethyl acetate. After concentration of the organic extract to dryness, the residue was purified by silica gel chromatography (silica gel SI 60, 63–200 µm, 30 × 1.5 cm; Merck), and linear gradient elution using dichloromethane as solvent A and methanol+acetic acid (99.9+0.1 by volume) as solvent B. The gradient was from 100% solvent A to 15% solvent B within 3 h at a flow rate of 3 ml min<sup>-1</sup> (LC Gradient Pump Mod. 2249; LKB). The fractions containing 1 and 2 were extracted with cold water to remove acetic acid and to precipitate daidzein. After concentration of the organic layer to dryness, the residue was dissolved in a small amount of methanol and purified on a Sephadex LH-20 column (90 × 2.5 cm) using methanol as the eluent. Pure metabolites were obtained by preparative reversed-phase HPLC using a stainless steel column (250 × 16 mm) filled with 10-µm Nu-

cleosil-100 C-18, and linear gradient elution with water+acetonitrile, starting from 40% acetonitrile to 70% acetonitrile over 15 min at a flow rate of 20 ml min<sup>-1</sup>. The preparative HPLC system consisted of two high-pressure pumps (Sepapress HPP-200/100, Kronlab), a gradient unit (Sepacon GCU-311), and a Valco preparative injection valve (6UW; VICI) with 5-ml sample loop. The UV absorbance of the eluate was monitored at 240 nm by a Gilson spectrophotometer Mod. 116 equipped with a preparative cell.

### 2.3 HPLC Analysis

The chromatographic system consisted of a HP 1090 M liquid chromatograph equipped with a built-in diode array detector, HP 79994B Pascal-workstation and HP 79988A software rev. 5.3 (Hewlett-Packard). Multiple wavelength monitoring was performed at 210, 230, 260, 280, 310, 360, and 435 nm without reference wavelength; the spectrum range was 200 to 600 nm in steps of 2 nm and sampling interval 640 ms.

A sample of the fermentation broth was centrifuged (10 min, 13 000 g), and 10 µl of the supernatant injected onto an HPLC column (125 × 4.6 mm), fitted with a guard column (20 × 4.6 mm), packed with 5-µm Nucleosil-100 C-18 (Grom). The samples were separated by linear gradient elution; solvent A was 0.1% phosphoric acid, solvent B was acetonitrile. The linear gradient was from 0% to 100% solvent B over 15 min with a 1 min hold at 100% B and a 5 min post time under initial conditions, at a flow rate of 2 ml min<sup>-1</sup>.

### 2.4 Structure elucidation

#### 2.4.1 Instrumentation

Infra-red spectra were recorded on sodium chloride using a Perkin-Elmer 1420 spectrophotometer. [<sup>1</sup>H] and [<sup>13</sup>C]NMR spectra were recorded at 600.13 MHz and 150.9 MHz in [D<sub>4</sub>]methanol (CD<sub>3</sub>OD) solution on a Bruker DMX 600 NMR spectrometer. Proton-detected heteronuclear correlations were measured using HMQC (optimized for <sup>1</sup>J<sub>HC</sub> = 150 Hz) and HMBC (optimized for <sup>n</sup>J<sub>HC</sub> = 7 Hz). EIMS: 70 eV (Finnigan MAT 8200).

#### 2.4.2 (2E,4Z)-Decadienoic acid (1)

IR  $\nu_{\max}$  2929, 1693, 1632, 1422, 1281, 1202 cm<sup>-1</sup>; [<sup>1</sup>H]NMR  $\delta$  0.91 (t,  $J$  = 7.1 Hz, 3H, 10-H), 1.32 (m<sub>c</sub>, 2H, 8-H), 1.33 (m<sub>c</sub>, 2H, 9-H), 1.44 (quin, 7.3 Hz, 2H, 7-H), 2.30 (qd,  $J$  = 7.5 Hz,  $J$  = 1.4 Hz, 2H, 6-H), 5.87 (d,  $J$  = 15.6 Hz, 1H, 2-H), 5.89 (dd,  $J$  = 10.6 Hz,  $J$  = 7.7 Hz, 1H, 5-H), 6.17 (dd,  $J$  = 11.1 Hz,  $J$  = 11.1 Hz, 1H, 4-H), 7.60 (dd,  $J$  = 14.3 Hz,  $J$  = 11.7 Hz, 1H, 3-H); [<sup>13</sup>C]NMR  $\delta$  14.38 (C-10), 23.54 (C-9), 29.06 (C-6), 30.19 (C-7), 32.51 (C-8), 123.23 (C-2), 127.70 (C-4), 140.73 (C-3), 142.26 (C-5), 171.21 (C-1); EIMS  $m/z$  (%): 168 (40) [M<sup>+</sup>], 139 (10) [M<sup>+</sup>-C<sub>2</sub>H<sub>5</sub>], 99 (65) [C<sub>5</sub>H<sub>7</sub>O<sub>2</sub><sup>+</sup>], 97 (100) [C<sub>5</sub>H<sub>5</sub>O<sub>2</sub><sup>+</sup>], 81 (39) [C<sub>5</sub>H<sub>5</sub>O<sup>+</sup>], 79 (38) [C<sub>6</sub>H<sub>7</sub><sup>+</sup>], 70 (36), 67 (65), 55 (52), 41 (76); HREIMS C<sub>10</sub>H<sub>16</sub>O<sub>2</sub> 168.1150 (calc), 168.1140 (exp).

### 2.4.3 (2E,4Z,7Z)-Decatrienoic acid (2)

IR  $\nu_{\max}$  2950, 1680, 1620, 1410, 1275, 990, 870  $\text{cm}^{-1}$ ;  $^1\text{H}$  NMR  $\delta$  0.98 (t,  $J=7.5\text{ Hz}$ , 3H, 10-H), 2.11 (quintd,  $J=7.5\text{ Hz}$ ,  $J=1.5\text{ Hz}$ ,  $J=0.8\text{ Hz}$ , 2H, 9-H), 3.05 (dd,  $J=7.6\text{ Hz}$ ,  $J=7.6\text{ Hz}$ , 2H, 6-H), 5.32 (dt,  $J=10.6\text{ Hz}$ ,  $J=7.3\text{ Hz}$ ,  $J=1.6\text{ Hz}$ , 1H, 7-H), 5.44 (dt,  $J=10.6\text{ Hz}$ ,  $J=7.3\text{ Hz}$ ,  $J=1.6\text{ Hz}$ , 1H, 8-H), 5.82 (dt,  $J=10.6\text{ Hz}$ ,  $J=7.9\text{ Hz}$ , 1H, 5-H), 5.87 (d,  $J=15.1\text{ Hz}$ , 1H, 2-H), 6.17 (dd,  $J=11.2\text{ Hz}$ ,  $J=11.2\text{ Hz}$ , 1H, 4-H), 7.64 (dd,  $J=14.8\text{ Hz}$ ,  $J=12.2\text{ Hz}$ , 1H, 3-H);  $^{13}\text{C}$  NMR  $\delta$  14.50 (C-10), 21.48 (C-9), 27.26 (C-6), 123.08 (C-2), 126.58 (C-7), 127.39 (C-4), 134.00 (C-8), 140.08 (C-5), 140.64 (C-3), 170.86 (C-1); EIMS  $m/z$  (%): 166 (6)  $[\text{M}^+]$ , 137 (7)  $[\text{M}^+-\text{C}_2\text{H}_5]$ , 123 (17)  $[\text{M}^+-\text{C}_3\text{H}_7]$ , 121 (45)  $[\text{M}^+-\text{COOH}]$ , 97 (40), 91 (48), 81 (33), 79 (100)  $[\text{C}_6\text{H}_7^+]$ , 77 (34); HREIMS  $\text{C}_{10}\text{H}_{14}\text{O}_2$  166.0994 (calc), 166.0994 (exp).

### 2.5 Test for herbicidal activity

Herbicidal activity was determined using *Lemna minor* L (duckweed) and *Lepidium sativum* L (garden cress) as test organisms. One hundred microliters of the test solutions in the respective organic solvent were pipetted into each well of a 24-well plate. After evaporation of the solvent, 1 ml of warm nutrition agar containing ( $\text{litre}^{-1}$ ):  $\text{KNO}_3$  (0.4 g),  $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$  (0.54 g),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.61 g),  $\text{KH}_2\text{PO}_4$  (0.2 g); trace element solution (1 ml),  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (9.8 mg), Na-EDTA (9.8 mg), and agar (15 g), was added. The trace element solution contained in deionized water ( $100\text{ ml}^{-1}$ ):  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  (5 mg),  $\text{MnCl}_2 \cdot 7\text{H}_2\text{O}$  (41.5 mg),  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$  (2.5 mg),  $\text{CuSO}_4 \cdot 7\text{H}_2\text{O}$  (2.5 mg),  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$  (12 mg), and  $\text{H}_3\text{BO}_4$  (50 mg). The pH was adjusted to 4.8 prior to autoclaving. After cooling of the agar either one young plant of *Lemna minor* having two leaves was deposited in each well on the surface of the medium, or a

*Lepidium sativum* seed was placed on the agar. Incubation times were three and five days in a climatic chamber with 16/8 h light/dark photoperiod, 24/20 °C day/night temperature and 60% humidity. Activity was evaluated by rating the ratio of bleaching and growth in the case of *Lemna minor*. The score was set to '0' for growth like the control, '2' for no growth, '5' for no growth and partial bleaching, '8' for total bleaching. Herbicidal activity against *Lepidium sativum* was measured as inhibition of germination of the seeds.

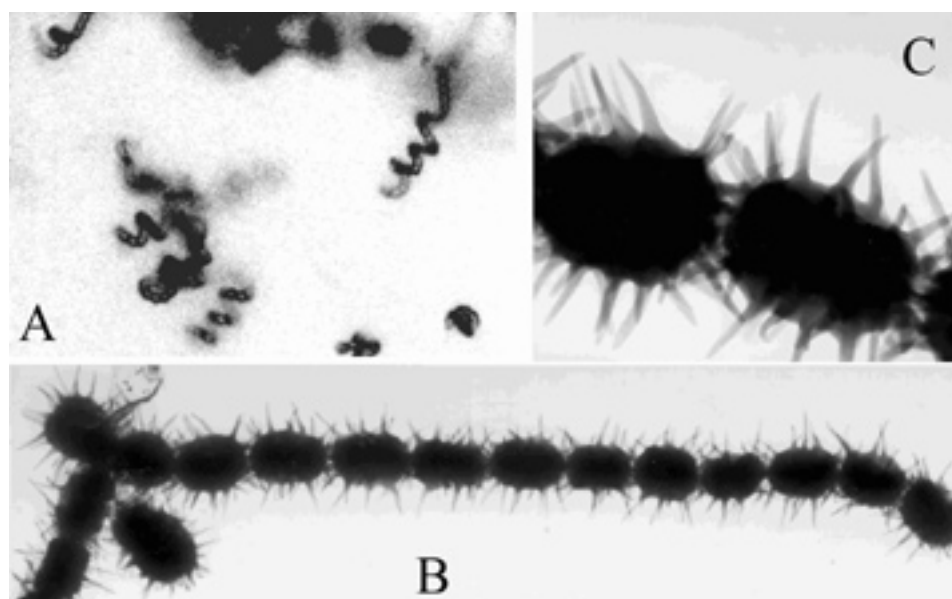
## 3 RESULTS

### 3.1 Taxonomy of the producing strain

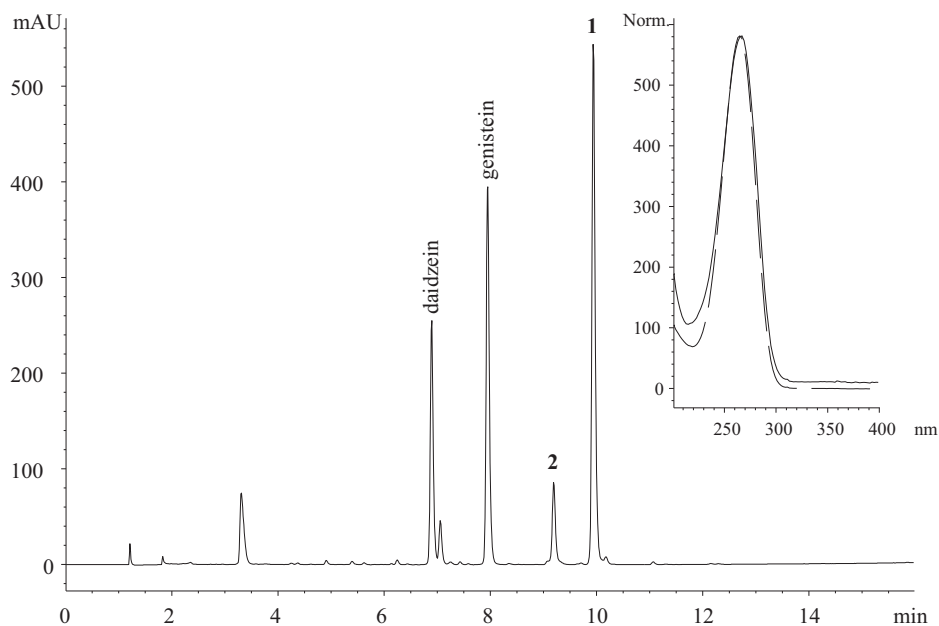
The chemotaxonomic analyses of diagnostic cell envelope compounds revealed strain Tü 6105 to be a member of the genus *Streptomyces* as characterized by the presence of LL-diaminopimelic acid in the peptidoglycan, by the 33:29:21:4 ratio of the menaquinones MK-9( $\text{H}_4$ ), MK-9( $\text{H}_6$ ), MK-9( $\text{H}_2$ ), MK-9( $\text{H}_8$ ), and by the typical pattern of saturated *iso*- and *anteiso*-branched fatty acids.

This genus affiliation was supported by morphological features. The spore mass colour of the aerial mycelium was greyish-blue. The spore chains were open spirals with two to six turns. They were generally long with more than 20 spores per chain. Long, straight flexuous spore chains (rectiflexibiles-type) were also observed. The spore surface was spiny as revealed by electron microscopy (Fig 2). The colour of the substrate mycelium varied from yellowish-brown through brown to olive-brown depending on the culture medium and the age of the culture. Melanoid pigments and soluble pigments were not produced.

On the basis of the results described above and the comparison of these properties including the utilization of selected carbon sources<sup>4</sup> with *Streptomyces viridochromogenes* DSM 40110<sup>T</sup>, the strain Tü 6105



**Figure 2.** Scanning electron micrograph of *Streptomyces viridochromogenes* Tü 6105. (A) spore chains (132 $\times$ ); (B) spore chain (7000 $\times$ ); (C) spores (40000 $\times$ ).



**Figure 3.** HPLC analysis of the culture filtrate extract of *Streptomyces viridochromogenes* Tü 6105 monitored at 260nm, and overlaid UV spectra of decadienoic acid (1) and decatrienoic acid (2).

was assigned to *S. viridochromogenes*. This affiliation is in agreement with the data in the literature.<sup>5,6</sup>

### 3.2 Diversity of metabolites

A crude extract of the culture filtrate from strain Tü 6105, grown in a medium consisting of mannitol (20g litre<sup>-1</sup>) and soybean meal (20g litre<sup>-1</sup>) was screened by the HPLC-DAD method and our HPLC-UV-Vis database.<sup>1</sup> Besides the isoflavones daidzein and genistein, two further metabolites 2 and 1, with retention times of 9.2min and 9.9min, respectively, were detected, having nearly congruent UV spectra with maxima at 266 and 265nm, respectively. Both spectra showed a high degree of similarity to that of the reference compound aranorosinol B, an antibiotic produced by the fungus *Pseudoarachnion roseus* H Kuehn (synonym *Gymnascella dankaliensis* (A Castellani) Currah),<sup>7</sup> by a match factor of 998. The maximum matching value is 1000 indicating identical spectra. However, the retention time of aranorosinol B (10.5min) differed from that of the metabolites from strain Tü 6105, therefore the compounds were not identical. The HPLC elution profile of the culture filtrate extract and UV spectra of 1 and 2 are shown in Fig 3.

When strain Tü 6105 was cultivated on a medium consisting of mannitol (20g litre<sup>-1</sup>) and cotton seed (20g litre<sup>-1</sup>), the metabolite pattern changed completely, and actinomycin D was identified by the HPLC-UV-Vis database as a minor compound.

### 3.3 Fermentation and isolation

Batch fermentations of *S. viridochromogenes* Tü 6105 were carried out in 20-litre stirred tank fermentors. Production of 1 and 2 began at about 8h and reached a maximum at 26h with a concentration of 29mg litre<sup>-1</sup>

1 and 7.7mg litre<sup>-1</sup> 2. Both compounds were metabolized rapidly and after 70h of fermentation time, neither 1 nor 2 could be detected in the culture filtrate.

The isolation of 1 and 2 required six purification steps. Polystyrene resin chromatography with Amberlite XAD-16 was used to reduce the culture filtrate volume and to enrich the metabolites, which were then extracted by ethyl acetate and purified by silica gel chromatography. After precipitation of daidzein, the raw product was subjected to exclusion chromatography with Sephadex LH-20. Finally, pure and separated compounds were obtained each in a yield of about 60% after preparative reversed-phase HPLC using Nucleosil C-18 material.

### 3.4 Structure elucidation

The first structural information about 1 was deduced from the HREIMS (molecular ion at  $m/z$  168.1140), which suggested the molecular formula C<sub>10</sub>H<sub>16</sub>O<sub>2</sub>. With the [<sup>13</sup>C]NMR spectrum, showing peaks at  $\delta$  171.21ppm (COOH) as well as  $\delta$  123.23, 127.70, 140.73, and 142.26ppm (C=C) and, therefore, indicating an unsaturated carbonic acid, the structure of a decadienoic acid was highly plausible. A conjugation of both double bonds to the carboxy group can clearly be seen from the chemical shifts of the protons attached to the double bonds ( $\delta$  5.87, 5.89, 6.17, and 7.60) and from the IR band at  $\nu$  1693cm<sup>-1</sup> ( $\alpha,\beta$ -unsaturated acid). This is confirmed by H,H-COSY measurements and, in particular, by HMBC correlations between 3-H ( $\delta$  7.60ppm) and C-1 (171.21), C-4 (127.70) and C-5 (142.26) as well as between 4-H ( $\delta$  6.17ppm) and C-2 (123.23) and C-3 (140.73) (Table 1), thus resulting in the structural proposal of 2,4-decadienoic acid.

The last pieces of structural information required

**Table 1.** Chemical shifts, HMQC, and HMBC correlations indicative of the constitution of metabolite **1**

Carbon	ppm	HMQC Corr	ppm	HMBC Corr	ppm
C-10	14.38	10-H	0.91	9-H	1.33
C-9	23.54	9-H	1.33	10-H	0.91
				8-H	1.32
				7-H	1.44
C-6	29.06	6-H	2.30	7-H	1.44
				5-H	5.89
C-7	30.19	7-H	1.44	6-H	2.30
				5-H	5.89
C-8	32.51	8-H	1.32	10-H	0.91
				9-H	1.33
				7-H	1.44
				6-H	2.30
C-2	123.23	2-H	5.87	4-H	6.17
C-4	127.70	4-H	6.17	3-H	7.60
				6-H	2.30
C-3	140.73	3-H	7.60	4-H	6.17
C-5	142.26	5-H	5.89	7-H	1.44
				6-H	2.30
				3-H	7.60
C-1	171.21			3-H	7.60

were the configurations at the double bonds. The coupling constants of  $c$  15 Hz between 2-H and 3-H on the one hand and  $c$  11 Hz between 4-H and 5-H on the other hand reveal a (2*E*,4*Z*) (2-*trans*-4-*cis*) configuration, thus establishing the structure of **1** as (2*E*,4*Z*)-decadienoic acid. The  $^1\text{H}$  NMR data match with those reported in the literature.<sup>8</sup> Physical data of the corresponding (2*E*,4*E*)-decadienoic acid from synthetic sources are found in Reference 9–14.

Compound **2** showed a HREIMS molecular ion at  $m/z$  = 166.0994, which indicates a molecular formula of  $\text{C}_{10}\text{H}_{14}\text{O}_2$ . This, in combination with the NMR and IR spectra, suggests a structure similar to that of **1**, differing only in the number of double bonds. The  $^{13}\text{C}$  NMR signals at 126.58 and 134.00 ppm and the  $^1\text{H}$  NMR signals at  $\delta$  5.44 and 5.32 ppm prove the existence of one further double bond that is in conjugation neither to the other two nor to the carboxy group. The HMBC correlations shown in Table 2 establish the additional double bond to be situated between C-7 and C-8. From the coupling constants between 7-H and 8-H (10.6 Hz) a *Z* (*cis*) configuration can be deduced for this double bond, whereas the configurations at the other double bonds are as in **1**. Therefore, compound **2** is proposed to be (2*E*,4*Z*,7*Z*)-decatrienoic acid. This compound has not previously been reported in the literature. For the physical data of the corresponding aldehyde, see References 15, 16.

### 3.5 Biological activity

Decadienoic acid (**1**) and decatrienoic acid (**2**) were tested for antimicrobial activity on a wide range of micro-organisms including Gram-negative and Gram-positive bacteria, yeasts and filamentous fungi in agar diffusion assays on complex and defined media. No

**Table 2.** Chemical shifts, HMQC, and HMBC correlations indicative of the constitution of metabolite **2**

Carbon	ppm	HMQC Corr	ppm	HMBC Corr	ppm
C-10	14.50	10-H	0.98	9-H	2.11
C-9	21.48	9-H	2.11	10-H	0.98
				8-H	5.44
				7-H	5.32
C-6	27.26	6-H	3.05	7-H	5.32
				5-H	5.82
				4-H	6.17
C-2	123.08	2-H	5.87	3-H	7.64
				4-H	6.17
C-7	126.58	7-H	5.32	8-H	5.44
				9-H	2.11
				6-H	3.05
C-4	127.39	4-H	6.17	6-H	3.05
				3-H	7.64
				2-H	5.87
C-8	134.00	8-H	5.44	10-H	0.98
				9-H	2.11
				7-H	5.32
				6-H	3.05
C-5	140.08	5-H	5.82	6-H	3.05
				3-H	7.64
C-3	140.64	3-H	7.64	4-H	6.17
C-1	170.86			3-H	7.64

antimicrobial activity could be detected in the case of **2**. Compound **1** showed weak activities against *Brevibacillus brevis* (Migula) Shida *et al.* ATCC 8246 and *Candida albicans* (Robin) Berkhout CBS 2718.

Both substances showed herbicidal activity against *Lemna minor*. The minimal inhibition concentration (MIC) was determined as the lowest concentration with complete bleaching of *L. minor*. A growth inhibition without bleaching was not observed. MIC of **2** was determined to be about 30  $\mu\text{g}$  per plant against *Lemna minor* and *Lepidium sativum*. **1** showed complete bleaching of *Lemna minor* down to 30  $\mu\text{g}$  per plant.

The herbicidal activity of the metabolites **1** and **2** isolated from *S viridochromogenes* Tü 6105 was compared with those of aliphatic fatty acids and structural

**Table 3.** Herbicidal activity of fatty acids and structural analogues on *Lemna minor*

	MIC [ $\mu\text{g}$ per plant]
Nonanoic acid	60
Decanoic acid	30–60
2,4-Decadienoic acid ( <b>1</b> )	30
2,4,7-Decatrienoic acid ( <b>2</b> )	30
1-Decanol	>60
2-Decanone	>60
9-Decen-1-ol	>60
Undecanoic acid	20
10-Undecenoic acid	30
1-Undecanol	>60
2-Undecanol	>60
2-Undecanone	>60

analogs with similar chain length. The results summarized in Table 3 show that the effect on *Lemna minor* was diminished when an alcohol or aldehyde group replaced the acidic group. The optimal chain length for activity was 11 carbon atoms. When polyunsaturated fatty acids were tested, the number of double bonds had no unequivocal effect on the herbicidal potency of the substances. **1** was more potent than the saturated acid, **2** showed similar effects to **1**. Undecanoic acid was slightly more active than 10-undecenoic acid.

#### 4 DISCUSSION

The herbicidal activity of fatty acids on a molecular basis is still not understood today. While the addition of saturated and unsaturated long-chain fatty acids (C<sub>16</sub>–C<sub>18</sub>) to quizalofop enhanced the phytotoxic effects of this herbicide, this was ascribed to increased absorption of the herbicide in the presence of the fatty acids rather than phytotoxic effects of the fatty acids themselves.<sup>17</sup>

Structurally related host-specific toxins having a 9,10-epoxy-9-methyl decatrienoic acid structure with a substituent in position 8 have been described from three pathotypes of *Alternaria*. This moiety was found in AK, AF and ACT toxins. It appears that these toxins have a primary effect on plasma membranes of susceptible cells.<sup>18</sup> For the herbicidal activity of **1** and **2** the chain length of C<sub>10</sub> or C<sub>11</sub> and the free acid group are important for the phytotoxic effect. It is assumed that, as in the case of the *Alternaria* phytotoxins, **1** and **2** affect the cytoplasmic membrane of the plant cells because of their lipophilic nature and free acidic group.

Undecanoic acid has been tested for additive or synergistic effects with the herbicide glufosinate-ammonium (Basta®; AgrEvo) in the *Lemna minor* assay. Mixing of the two substances below their MICs (undecanoic acid 4.4 µg, glufosinate-ammonium 0.4 µg) did not result in a herbicidal effect (results not shown).

(2*E*,4*Z*)-Decadienoic acid (stilingic acid, **1**) has previously been isolated from stillingia oil from the seeds of *Sapium sebiferum* (L) Roxb (Euphorbiaceae).<sup>14,19</sup> No biological activity has been reported for this compound. The corresponding (2*E*,4*Z*)-decadiendioic acid (phycodioic acid) is known as a metabolite from the zygomycete *Phycomyces blakesleeanus* Burgeff, but again no biological activity has been recorded.<sup>20</sup> Its methyl ester is a pheromone of *Pityogenes chalcographus* L (Coleoptera),<sup>21,22</sup> and the ethyl esters were detected as the odoriferous principle for Bartlett pears (*Pyrus communis* L, Rosaceae).<sup>23,24</sup> To our knowledge, **1** has not been shown to have any antibiotic activity nor has it ever been isolated from bacteria before. (2*E*,4*Z*)-decadienamides has been isolated from *Amycolatopsis* sp. It has been named amidenin and was found during a screening for plant growth regulating substances from actinomycetes.

Amidenin reduced the damage to rice plants caused by some herbicides.<sup>25</sup> While amidenin exhibited no insecticidal activity, the *N*-isobutyl-(2*E*,4*E*)-decadienamide from *Anacyclus pyrethrum* (L) Link and *Achillea millefolium* L were found to have strong insecticidal and larvicidal effects.<sup>26</sup>

(2*E*,4*Z*,7*Z*)-Decatrienoic acid (**2**) has up to now not been described as a natural product nor has any biological activity been published previously. The structurally related (2*E*,4*E*,6*Z*)-decatrienoic acid was reported as a constituent of triterpene esters in the latex of *Euphorbia pulcherrima* Willd (Euphorbiaceae),<sup>27</sup> and several derivatives of (2*E*,4*E*,6*Z*)-decatrienoic acid dehydropiperidide were isolated from various *Achillea* spp (Compositae).<sup>28</sup>

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